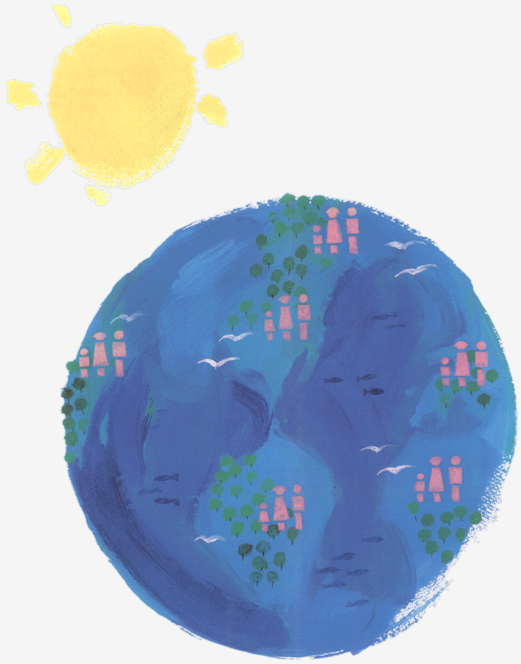
NICEATM

National Toxicology Program Center for the Evaluation Of Alternative Methods

ICCVAM

Interagency Coordinating Committee on the Validation of Alternative Methods

Proposed Reference Substances
BRD Reanalysis Summary



Expert Panel Public Teleconference
September 19, 2005
Research Triangle Park, NC

National Toxicology Program

NIEHS

National Institutes of Health

Department of Health & Human Services • USA

Proposed Reference Substances are Intended to:

- Represent a range of ocular responses
- Represent a range of chemical/product classes
- Represent a range of known or anticipated mechanisms or modes of action
- Be based on high quality *in vivo* rabbit eye test method studies
- Have a well-defined chemical composition
- Have been tested at a defined concentration and purity
- Be readily available

Expert Panel Recommendations and Changes to the Reference Substances List

- Increase the number of inorganic substances
  - *Number of inorganics increased from 2 to 11*
- Include substances that are known human ocular corrosives or severe irritants, even in the absence of *in vivo* rabbit data
  - *10 human ocular corrosives/irritants added*
- Remove formulations
  - *Formulations removed*
- Reduce number of surfactants
  - *Number of surfactants decreased from 12 to 7*
- Decrease the total number of proposed reference substances from 89
  - *Number of proposed reference substances increased*

Minimum Number of Substances Needed to Evaluate Accuracy

- Number of substances needed to evaluate the accuracy of an alternative test method will vary depending on:
  - The range of possible responses that the test method is expected to be able to measure
  - The diversity of the known or anticipated mechanisms or modes of action that are involved in producing a toxic response
  - The number of chemical/physical classes and physicochemical properties that the test method is expected to be able to evaluate
- Preliminary statistical evaluation indicates that several hundred substances could potentially be required to evaluate the accuracy of a test method with a high level of confidence

Properties Needed in Reference Substance List

- For the detection of ocular corrosives and severe irritants, the list of substances needs to include substances that:
  - Induce very severe responses within a relatively short period, as well as those where the response is delayed
  - Adversely affect the cornea, iris, and/or conjunctiva
  - Induce persistent and non-persistent lesions
  - Represent a diverse population of chemical classes and physicochemical properties

Revised Proposed Reference Substances List

- To meet these needs, the list was increased from 89 substances to 122 substances
- The list includes:
  - 79 GHS Category 1 substances
    - 10 substances classified based on human data
  - 28 GHS Category 2 substances
  - 15 GHS nonirritant substances
  - 34 chemical classes
  - 29 product classes
  - 79 liquids
  - 43 solids

Distribution of Substances in the Proposed Reference Substances (by GHS Classification)

GHS Class-ification	Entries in the *In Vivo* Database[1] (Revised/ Original)	Candidate Substances (Revised/ Original[2])	Proposed Reference Substances (Revised/ Original)	Additional Substances (Human)	Final Number of Proposed Reference Substances (Revised/ Original)
Category 1	220/123	93/48	69/48	10	79/48
Category 2A	62/24	17/11	15/11	NA	15/11
Category 2B	51/68	23/27	13/15	NA	13/15
Nonirritant	497/277	77/111	15/15	NA	15/15
Total	**830/492**	**210/197**	**112/89**	**10**	**122/89**

Abbreviation: GHS = Globally Harmonized System (UN [2003]); NA = Not applicable.

[1]The complete database includes multiple entries for some substances, as well as formulations, coded substances, and substances that could not be classified according to the GHS ocular hazard classification system.

[2] The number of entries decreased for some GHS classification categories due to (1) the reclassification of some substances as GHS Category 1 irritants, based on the persistence of any lesion to day 21 post-treatment; (2) a reassessment of current commercial availability; and (3) collapsing multiple studies with the same substance tested at the same concentration into a single entry.

NICEATM-Defined Subcategories for the Proposed GHS Category 1 Substances

Subcategory	Criteria for Classification as a GHS Category 1	Number of Substances (Revised/ Original)
0[1]	Not Classifiable	12/0
1	Positive response based on a persistent lesion involving the cornea, iris, and/or conjunctiva through day 21 in at least one of three rabbits and not on severity	9/18
2	Positive response based on mean for first 3 days (CO score >3 and <4 or IR score >1.5) in at least two of three rabbits but lesions do not persist through day 21	4/4
3	Positive response based on mean for first 3 days (CO score >3 and <4 or IR score >1.5) in at least two of three rabbits and a persistent (>21 days) lesion in at least one rabbit	4/2
4	CO score = 4 at any time in at least one of three rabbits	50/24
	Total	**79/48**

Abbreviations: CO = Corneal opacity; GHS = United Nations Globally Harmonized System (UN [2003]), IR = Iritis.

[1]Included are two GHS Category 1 substances that could not be subclassified because classification was based on an extreme response shortly after treatment in the only animal tested and 10 substances classified as GHS Category 1 irritants because they induced a severe ocular response in accidentally-exposed humans, and appropriate *in vivo* rabbit ocular irritancy test data was not located for these 10 substances.